Please change the Title of the Invention from "Rapid Detection And Identification Of Pathogens" to --Methods for modifying an oligonucleotide--.

IN THE CLAIMS:

Please cancel Claims 1-44.

Please add the following Claims:

45. A method for modifying an oligonucleotide, said method comprising:

(a) combining said oligonucleotide with a polynucleotide and a 5'-nuclease, said oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5' portion which does not hybridize to the polynucleotide,

(b) incubating said oligonucleotide, said polynucleotide, and said nuclease under isothermal conditions, whereby a duplex formed by hybridization of the 3' portion of the oligonucleotide to the polynucleotide is in equilibrium with unhybridized oligonucleotide and unhybridized polynucleotide, said isothermal conditions being at or near the melting temperature of said complex, and

(c) while maintaining said isothermal conditions, cleaving said oligonucleotide with said nuclease when said 3'-portion is hybridized to said polynucleotide to provide:

(i) a first fragment including said 5'-portion and no more than one nucleotide from the 5' end of said 3'-portion, and

(ii) a second fragment that is 3' of said first fragment with reference to the intact oligonucleotide, thereby modifying said oligonucleotide, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

46. The method of claim 45, wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

47. The method of claim 45, further comprising incubating a second oligonucleotide under said isothermal conditions with said oligonucleotide, said

polynucleotide, and said 5'-nuclease, wherein said second oligonucleotide substantially non-reversibly hybridizes under said isothermal conditions to a site on said polynucleotide that is in the 3' direction from the site at which said oligonucleotide hybridizes.

48. The method of claim 47, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3 °C higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

49. A method for amplifying a signal associated with the presence of a polynucleotide analyte, said method comprising:

(a) providing in combination a polynucleotide analyte, a 5'-nuclease and a molar excess, relative to the concentration of said polynucleotide analyte, of an oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to said polynucleotide,

(b) under isothermal conditions, establishing an equilibrium between said oligonucleotide, said polynucleotide analyte, and a duplex formed by the hybridization of the 3' portion of said oligonucleotide with said polynucleotide analyte, said isothermal conditions being at or near the melting temperature of said duplex,

(c) while maintaining said isothermal conditions, cleaving said oligonucleotide with said 5'-nuclease when said oligonucleotide is hybridized to said polynucleotide to provide,

(i) a first fragment including said 5'-portion and no more than one nucleotide from the 5'-end of said 3'-portion, and

(ii) a second fragment including at least one of said 3' portion and said 3' portion lacking one nucleotide, wherein at least one of said first fragment and said second fragment generates a signal, and

(d) while maintaining said isothermal conditions, maintaining said equilibrium to amplify the amount of at least one of said first fragment and said second fragment and thereby amplifying said signal, wherein said first fragment and

said second fragment are continuously produced under said isothermal conditions.

50. The method of claim 49 further comprising maintaining said equilibrium until at least a 100-fold molar excess of said first fragment and/or said second fragment are obtained relative to the molar amount of said polynucleotide analyte.

51. The method of claim 49 wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaciae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanasomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobaccillus moniliformis, Donvania granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

52. The method of claim 49, further comprising hybridizing a second oligonucleotide to said polynucleotide analyte under said isothermal conditions, wherein said second oligonucleotide hybridizes to a site on said polynucleotide analyte that is in the 3' direction of the site at which said oligonucleotide hybridizes, and wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3°C higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

53. The method of claim 49, wherein said oligonucleotide hybridization sites are contiguous.

54. The method of claim 49, wherein at least one of said first fragment and said second fragment has a label.

55. The method of claim 54, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

56. A method for detecting a polynucleotide analyte, said method comprising:

(a) providing in combination a medium suspected of containing said polynucleotide analyte, a molar excess, relative to the suspected concentration of said polynucleotide analyte, of a first oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5'-portion which does not hybridize to said polynucleotide, a 5'-nuclease, and a second oligonucleotide that hybridizes to a site on said polynucleotide analyte in the 3'-direction of the site at which said first oligonucleotide hybridizes,

(b) under isothermal conditions, establishing an equilibrium between a complex formed by the hybridization of the 3' portion of said first oligonucleotide and said polynucleotide analyte, said polynucleotide analyte and said first oligonucleotide, said isothermal conditions being at or near the melting temperature of said complex, and wherein said second oligonucleotide is substantially fully hybridized to said polynucleotide analyte under said isothermal conditions,

(c) while maintaining said isothermal conditions, cleaving said first oligonucleotide when hybridized to said polynucleotide analyte with said 5'-nuclease to provide,

(i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte, and

(ii) a second fragment that is 3' of said first fragment in said first oligonucleotide and which substantially hybridizes to said polynucleotide analyte; and

(d) while maintaining said isothermal conditions, detecting the presence of said first fragment, said second fragment, or said first fragment and said second fragment, the presence thereof indicating the presence of said polynucleotide analyte

wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

57. The method according to claim 56, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3°C. higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

58. The method of claim 56, wherein said first fragment and/or said second fragment has a label.

59. The method of claim 58, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

60. The method of claim 56 wherein said polynucleotide analyte is DNA.

61. The method of claim 56, wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that portion of said first oligonucleotide that is capable of hybridizing to said polynucleotide analyte.

62. The method of claim 56, wherein said second oligonucleotide hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first oligonucleotide hybridizes.

63. The method of claim 56, wherein said first oligonucleotide has a substituent that facilitates separation of said first fragment or said second fragment from said medium.

64. The method of claim 56 wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaciae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanasomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobaccillus moniliformis, Donvania granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

65. A method for detecting a polynucleotide analyte, said method comprising:

(a) providing in combination a medium suspected of containing said polynucleotide analyte, a first oligonucleotide at least a portion of which reversibly hybridizes with said polynucleotide analyte under isothermal conditions to form a complex, said isothermal conditions being at or near the melting temperature of said complex, a 5'-nuclease, and a second oligonucleotide that hybridizes to a site on said polynucleotide analyte that is in 3' of, and contiguous with, the site at which said first oligonucleotide hybridizes, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is at least 3°C higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide,

(b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first oligonucleotide, wherein said first oligonucleotide, when hybridized to said polynucleotide analyte, is cleaved by said 5'-nuclease as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte,

(i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte, and

(ii) a second fragment that is 3' of said first fragment in said first oligonucleotide and which substantially hybridizes to said polynucleotide analyte; and

(c) detecting the presence of said first fragment, said second fragment, or said first fragment and said second fragment, the presence thereof indicating the presence of said polynucleotide analyte, wherein said polynucleotide analyte is from a source selected from the group consisting of Corynebacteria, Pneumococci, Streptococci, Staphylococci, Neisseria, Enterobacteriaciae, Enteric bacilli, Hemophilus-Bordetella, Pasteurellae, Brucellae, Aerobic Spore-forming Bacilli, Anaerobic Spore-forming Bacilli, Mycobacteria, Actinomycetes, Spirochetes, Trypanasomes, Mycoplasmas, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Streptobaccillus moniliformis, Donvania granulomatis, Bartonella bacilliformis, Rickettsiae, Adenoviruses, Herpes Viruses, Pox Viruses, Picornaviruses, Myxoviruses, Arboviruses, Reoviruses, Retroviruses, Fungi, Hepatitis Viruses, and Tumor Viruses.

66. The method of claim 65, wherein at least one of said first fragment and said second fragment has a label.

67. The method of claim 66, wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups, and suspendible particles.

68. The method of claim 65, wherein said polynucleotide analyte is DNA.

69. A method for modifying an oligonucleotide, said method comprising:

(a) combining said oligonucleotide with a polynucleotide and a 5'-nuclease, said oligonucleotide having a 3' portion capable of reversibly hybridizing to said polynucleotide and a 5' portion which does not hybridize to the polynucleotide,

(b) incubating said oligonucleotide, said polynucleotide, and said nuclease under isothermal conditions, and

(c) while maintaining said isothermal conditions, cleaving said oligonucleotide with said nuclease when said 3'-portion is hybridized to said polynucleotide to provide:

(i) a first fragment including said 5'-portion and no more than one nucleotide from the 5' end of said 3'-portion, and

(ii) a second fragment that is 3' of said first fragment with reference to the intact oligonucleotide, thereby modifying said oligonucleotide.

70. The method of claim 69, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

71. The method of claim 69, wherein the amounts of fragments that are formed are larger than the amount of said polynucleotide.

72. The method of claim 69, further comprising incubating a second oligonucleotide, said polynucleotide, and said 5'-nuclease, wherein said second oligonucleotide hybridizes to a site on said polynucleotide that is in the 3' direction from the site at which said oligonucleotide hybridizes.

73. The method of claim 72, wherein the melting temperature of the second oligonucleotide when hybridized to the polynucleotide is higher than the melting temperature of the first oligonucleotide when hybridized to the polynucleotide.

74. The method of claim 69 wherein said polynucleotide is from a pathogenic organism.

75. The method of claim 69, wherein at least one of said first fragment and said second fragment has a label.

76. A kit for detection of a polynucleotide comprising in packaged combination:

(a) a first oligonucleotide having the characteristic that, when reversibly hybridized under isothermal conditions to at least a portion of said polynucleotide, it is degraded by a 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and (ii) a second fragment that is 3' of said first